A novel ultrasound-based vascular calcification score (CALCS) to detect subclinical atherosclerosis

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Abstract. – **OBJECTIVE:** To quantify non-coronary vascular calcifications (VC) in asymptomatic patients at low-intermediate cardiovascular risk by a new color Doppler ultrasound (DUS)-based score (the carotid, aortic, lower limbs calcium score, CALCs), and to correlate this score with classical parameters associated with cardiovascular risk [carotid intima media thickness (IMT), and arterial stiffness (AS)].

PATIENTS AND METHODS: All consecutive asymptomatic patients who underwent a screening DUS of non-coronary circulation were evaluated and patients at low-intermediate cardiovascular risk were selected according to Framingham risk score (FRS).

Among them, we enrolled 70 patients with US evidence of VC and 71 age, sex and FRS matched controls. The presence of VC was correlated with classical markers of cardiovascular risk, such as AS and intima-media thickness (IMT). AS, expressed as pulse wave velocity (PWV) and arterial distensibility, carotid IMT and CALCs were measured for both groups. AS and c-IMT were assessed by a new Radio-Frequency (RF) DUS-based method. CALCs was generated by our previously described B-mode DUS-based method according to number/size of VC in 11 non-coronary segments (range 0-33).

RESULTS: Patients with VC presented higher AS and IMT values than controls (PWV 8.34±0.98 m/s vs. 6.74±0.68 m/s, *p*<0.0001; arterial distensibility 267±12 mm vs. 315±65 mm, *p*=0.001; IMT 687±132 mm vs. 572±91 mm, *p*<0.0001). Mean CALCs of patients with VC was 8.41±7.78. CALCs were significantly correlated with c-IMT (*p*<0.0001; r=0.3), PWV (*p*<0.0001; r=0.4) and arterial distensibility (*p*=0.002; r=-0.1).

CONCLUSIONS: DUS-based CALCs is highly correlated with other validated markers of subclinical atherosclerosis, such as c-IMT and AS. Our results demonstrated the ability of CALCs to identify individual predictive factors beyond the traditional risk factors by quantifying an interesting and novel step of the atherogenic process. Future studies on larger series and with adequate follow up are necessary to confirm these results and to evaluate the role of this new marker in monitoring calcific atherosclerosis progression.

Key Words:
CALCs, Vascular calcification, Calcific atherosclerosis, Atherosclerosis markers, Cardiovascular risk, Ultrasonography.

Introduction

Cardiovascular disease (CVD) is a major health problem in Western Countries, due to its slow and often asymptomatic progression, and its unavoidable impact on morbidity and mortality[1]. Calcific atherosclerosis (CA) has been associated with an increased prevalence of cardiovascular events, and can unexpectedly be observed in middle age or even in young subjects[2-5]. In order to prevent cardiovascular events in later life, there is an increasing interest in discovering new markers of atherosclerosis, which may be useful in providing an earlier diagnosis of CVD[6]. Subclinical inflammation, oxidative stress, and, particularly, loss of calcium homeostasis involving an impaired "bone-vascular crosstalk" have been recognized as important mechanisms underlying the progressive natural history of CVD[7]. Recent studies have advocated the pathologic deposition of calcium in the vessel wall with the simultaneous reduction of calcium deposition in the bone, the so called "calcium paradox", to explain CA. This process is related to a subclinical deficiency of Vitamin K2, alongside several other possible mechanisms[9]. Furthermore, many studies have underlined the high prognostic significance of VC.

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Calcifications in the intima and media tunica of coronary arteries (coronary artery calcification, CAC) can be detected by computed tomography (CT) that is able to provide a score based on calcium quantification[10]. It has been demonstrated that the CAC score is related to the prevalence of new cardiovascular events and CVD mortality. Moreover it seems to be superior to the Framingham risk score (FRS), stress echocardiography or perfusion studies in the identification of both high-risk asymptomatic subjects and older patients[11-15]. However, a notable limitation of the CAC score, as of other CT-imaging based scores, is that CT follow-up cannot be performed for primary prevention due to the risk related to radiation exposure as well as to the economic impact on public health expenditure[16,17]. Thus, other studies have focused on the quantification of peripheral artery calcification by cheaper and safer techniques; however, none of these have been performed in asymptomatic subjects. Adragao et al[18] found that in 101 hemodialysis patients, a simple vascular calcification score (SVCS) measured by plain X-ray of the pelvis and hands was associated with arterial stiffness, measured by pulse wave velocity (PWV) and pulse pressure (PP). Higher SVCS, PWV, and PP, were also shown to be associated with higher rates of mortality[19]. Although these results seem promising as they are based on simple and inexpensive methods, they do have some limitations: firstly, the score was calculated by examining only the ileo-femoral, radial and digital arteries; secondly, the presence of calcification was not graded according to severity; lastly, the maximum vascular calcification score was relatively low, ranging from 0 to 8. Another X-ray based score evaluating lumbar aortic calcifications was described by Kauppila et al[20] in 617 participants of the Framingham heart study. Although no correlation with vascular stiffness was provided, aortic calcification severity was graded using a 0-3 scale for each lumbar segment, and the final results were merged to develop four different composite scores. However, again the use of radiation exposure represents a recognizable limitation of the previously mentioned studies.

Therefore, there is an unquestionable need to develop new modalities for the detection and quantification of VC, which can be safely used in subjects with subclinical atherosclerosis. The "ideal" method should be non-invasive, repeatable, less expensive and more comprehensive than the previous modalities. The final goal was to correctly identify subjects with low-intermediate cardiovascular risk beyond the use of traditional risk factors, and ultimately to improve their prognosis. Among available non-invasive techniques, color Doppler Ultrasound (DUS) has previously been used to quantify calcium overload in patients with peripheral artery disease with promising results[21].

The aim of the present study was to assess the presence of calcifications in non-coronary arterial circulation in asymptomatic subjects at low-intermediate cardiovascular risk and to develop a new DUS-based calcification score, the carotid, aortic and lower limbs calcification score (CALCs). A secondary aim was to correlate this score with classical markers of subclinical atherosclerosis, such as intima media thickness (IMT) or arterial stiffness (AS)[22,23].

Patients and Methods

From December 2012 to January 2017, all consecutive outpatients who underwent a screening color DUS of non-coronary circulation at the Angiology Unit of the Catholic University of the Sacred Heart, Rome Italy were evaluated. Exclusion criteria were history of cardiovascular events, diabetes mellitus, uncontrolled hypertension, LDL-cholesterol>160 mmHg, active smoking (>10 cigarettes a day), GFR<60 ml/min, treatment with calcium, calcitriol or Vitamin K antagonists (VKA).

The FRS based on age, race, brachial artery pressure, total and HDL cholesterol, active smoking and presence of diabetes was calculated for all patients and used to select patients at low-intermediate cardiovascular risk.

Subjects without any evidence of VC matched for age, sex and FRS by propensity score were selected as a control group.

Both patients and matched controls underwent IMT, AS and CALCs measurement.

The study was performed in agreement with the Declaration of Helsinki and subsequent amendments. Written informed consent was obtained from all subjects and the study was approved by the Catholic University of the Sacred Heart Ethics Committee, Rome, Italy.

The presence and grade of vascular calcification were measured by DUS in 11 vascular segments (common carotid arteries, common femoral arteries, popliteal arteries, posterior tibial arteries, anterior tibial arteries, and abdominal aorta), according to a previously described method[22]. In

brief, one point was given for a single intimal or medial calcification with a thickness of less than 0.5 mm in a longitudinal scan (microcalcification, mC), 2 points for more than 1 mC in the same vascular segment, and 3 points for any calcification thicker than 0.5 mm (macrocalcification, MC). The final sum of points for each vascular segment generates the CALC score, ranging from 0 to 33 points.

Arterial stiffness was assessed by applying an automated radiofrequency-based method (Quality Arterial Stiffness [RF-QAS]; Esaote Medical Systems, Genoa, Italy) to the DUS examination of the left common carotid artery. Local pulse-wave velocity (PWV) was calculated by combining arterial distensibility with local distending pressure measurements. Assuming a constant difference between mean arterial pressure and diastolic pressure along the arterial tree, the QAS system can detect systo-diastolic changes in the arterial diameter following arterial wall movements during the cardiac cycle and converts local distension variations in modifications of local distending pressure (pulse pressure). To preserve measurement quality, the maximal distensibility of the left common carotid artery, computed in 6 cardiac cycles, was recorded only if the standard error was lower than 30 µm. PWV was calculated by the Bramwell-Hill equation, as follows: $PWV = \sqrt{\Delta P \cdot V / \Delta V \cdot \rho}$ where ΔV and ΔP represent changes in volume and pressure respectively, and ρ is blood density. Based on this equation PWV increases when there is an increase in arterial stiffness[24].

Intima-media thickness (IMT) was measured using the same radiofrequency-based technology outlined above (Quality Intima-Media Thickness [RF-QIMT]; Esaote Medical Systems, Genoa, Italy) on the left common carotid artery, i.e. 1 cm from the bifurcation. As for AS, to preserve measurement quality, mean IMT values, calculated over 6 cardiac cycles, were recorded only if the standard error was lower than 20 µm.

Statistical Analysis

A preliminary data analysis was performed to verify correct matching of patients and controls according to age, sex and FRS. Propensity scores allowed the inclusion of all patients/controls in the analysis except two who failed any matching possibility.

Non-parametric tests were adopted for statistical analysis due to the non-Gaussian distribution of variables. Kendall's Tau was used to investigate the correlation between CALCs, age, sex, FRS, and flow metric and morphologic vascular parameters (IMT, PWV, CCA distensibility). In order to determine which model fitted best with data distribution, the relationship between CALCs and each correlated variable was graphically explored, and linear regression was chosen. Finally, all variables were included in a multiple regression model to determine the factors independently associated with CALCs.

Continuous variables were expressed as a median and range, and categorical variables were reported as frequencies and percentages. p-value < 0.05 was considered statistically significant. Statistical analysis was performed using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria), version 3.4.0.

Results

Over a fifty-month period all patients referred to the Angiology Unit of Gemelli Foundation Hospital, Rome, Italy, to undergo DUS were screened. Among them, 885 patients at low-intermediate cardiovascular risk were enrolled according to selection criteria. Based on DUS evaluation, 70 patients (34 males and 36 females; mean age 54 ± 11 years) with intima or media tunica VC and 71 controls matched for age, sex and FRS (35 males and 36 females; mean age 53 ± 9 years) were included in the final statistical analysis.

The main demographic and clinical parameters of the patients are listed in Table I. Patients VC and controls. Conversely, patients with VC had higher AS (PWV: 8.34±0.98 m/s *vs.* 6.74±0.68 m/s, *p*<0.0001; CCA distensibility 316±65 µm *vs.* 267±12 µm, *p*=0.001) and IMT values (687±132 µm *vs.* 572±91 µm, *p*<0.0001) than controls.

The mean CALCs of patients with VC was 8.41±7.78. CALCs was directly correlated with IMT and PWV (tau 0.382 *p*<0.0001 and tau 0.475 *p*<0.0001 respectively), whereas, as expected, an inverse correlation with CCA distensibility was observed (tau -0.258 *p*=0.0001). Regression models confirmed these trends and showed a linear association between CALCs and DUS (CCA distensibility: beta 0.066 *p*=0.002; PWV: beta 0.380 *p*<0.0001; IMT: beta 0.280 *p*<0.0001; Figures 1-3). Finally, in order to define which factor was independently associated with the CALCs, a linear regression model including all DUS parameters (IMT: beta, PWV and CCA distensibility) was created. Only IMT and PWV were confir-

Table I. Demographic and clinical characteristics of patients with vascular calcifications (VC) and controls expressed as frequency or mean ± standard deviation (SD).

Parameter	Patients with VC Frequency/Mean ± SD	Controls Frequency/Mean ± SD	p-value
Sex	34 M/36 F	35 M/36 F	0.989
Age	54 ± 11	53 ± 9	0.366
Framingham Score	6 ± 3	5 ± 3	0.746
IMT	687 ± 132	572 ± 91	<0.0001
PWV	8.34 ± 0.98	6.74 ± 0.67	<0.0001
CCA distensibility	266 ± 65	346 ± 115	=.001
CALCs	8.41 ± 7.78	-	-

IMT, Intima Media Thickness; PWV, Pulse Wave Velocity; CALCs Carotid, aortic and lower limbs calcification score.

med to be directly and independently associated with CALCs (IMT 0.320 *p*<0.0001; PWV: beta 0.502 *p*<0.0001; CCA distensibility: beta 0.031 *p*=0.711; global *p*-value of the model <0.0001; Figure 4).

Discussion

CA plays a pivotal role in the development of cardiovascular disease, which is the main cause of morbidity and mortality in developed countries. Quantification of risk and evaluation of subclinical organ damage at an early stage are important to prevent acute and chronic complications and to increase patients' survival. In this paper we describe a new method to assess vascular calcification burden in the non-coronary circulation and its ability to evaluate subclinical organ damage, in addition to standard markers of subclinical atherosclerosis such as IMT and AS[25].

Our study focused on asymptomatic middle-aged subjects who represent the target population for preventive intervention. For this reason it is crucial to detect the earliest signs of cardiovascular disease and to develop new markers for stratifying patients according to their cardiovascular risk in addition to the currently known traditional risk factors.

The score that we propose as a new marker of subclinical vascular damage, CALCs, is simple, easy to perform and can be calculated in an estimated time of 5 min. It requires DUS, which is safer and relatively cheaper than other currently available imaging techniques (e.g. CT scan or Rx plan). Compared to CT-scan, CALCs is more cost-effective and can reach a high-level of accuracy if performed by expert operators.
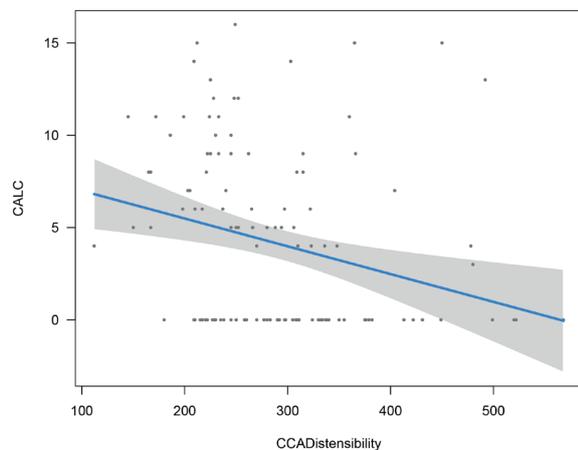
Figure 1. Correlation between CALCs and CCA distensibility. CALCs, carotid aortic and lower limbs calcification score. CCA, common carotid artery.
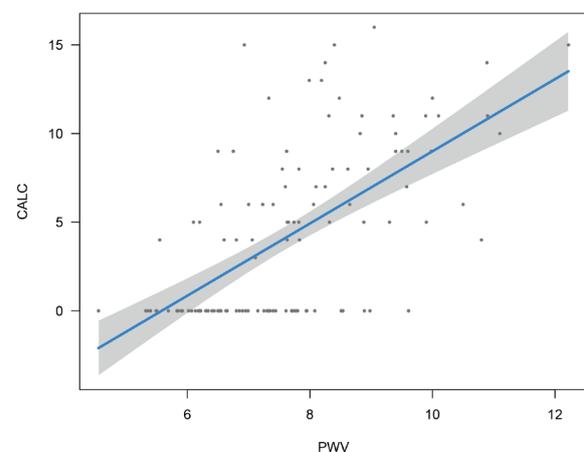
Figure 2. Correlation between CALCs and PWV. PWV, Pulse Wave Velocity; CALCs, carotid, aortic and lower limbs calcification score.
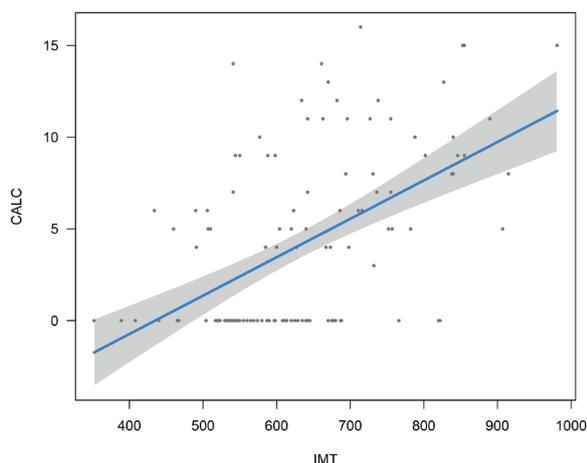
Figure 3. Correlation between CALCs and IMT. IMT, Intima Media Thickness; CALCs, carotid aortic and lower limbs calcification score.

Compared to other US-based scoring systems, CALCs comprises the evaluation of different arterial districts and allows a wide range of classifications, which are important to provide a more detailed stratification of pa-

tients. The sequential evaluation of 11 single segments of 4 cm each, selected based on a higher presence of calcifications detectable according to well-defined anatomic points, makes it possible to reach high-level of accuracy and reproducibility. Finally, CALCs can evaluate the severity of VC in each segment, with a final score range wide enough (from 0 to 33 points) to monitor negative evolution or even improvements of VC.

As the present study did not include patients affected by diabetes or chronic renal failure, due to higher expected rates of VC, the mean CALCs of the enrolled patients did not reach particularly high values, ranging from 3 to 16. In this setting, CALCs is mostly generated by mC (points 1 and 2) in the wall of popliteal and tibial arteries. MC within atherosclerotic plaques is present in less than 5%. However, we demonstrated the direct comparability of this score with classical markers of subclinical atherosclerosis, such as IMT and AS, measured by the best technology available. It is feasible that increased AS and VC may co-exist in the same subject, as expression of different stages of CA.
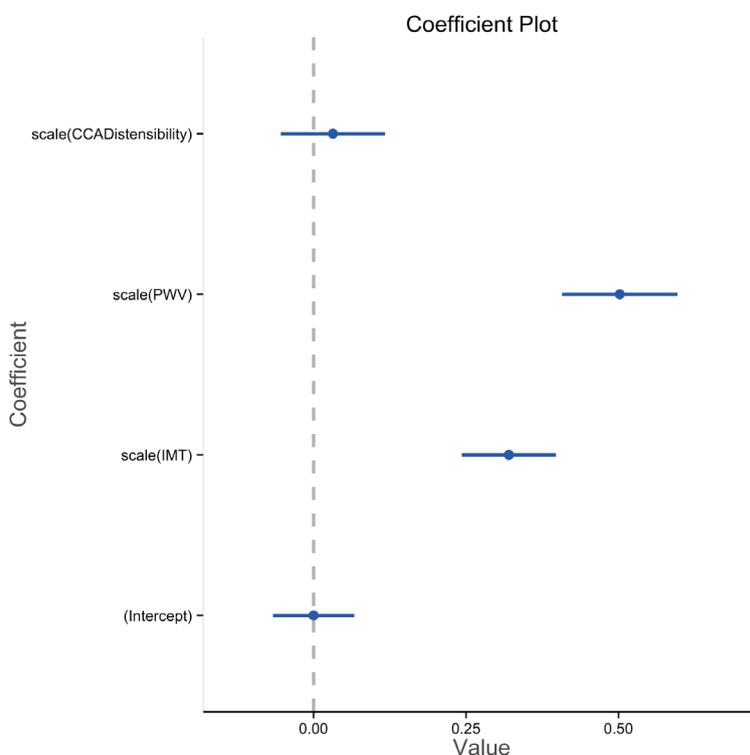
Figure 4. Coefficient plot from multivariable regression model including IMT, PWV and CCA distensibility. PWV, Pulse Wave Velocity; AD, Arterial Distension; CALCs, carotid aortic and lower limbs.

In this scenario, a positive CALCs may represent an adjunctive marker of atherosclerosis, besides the increase in AS and IMT.

We found a CALCs >2 in up to 7.9% of our "healthy" examined subjects. This is not surprising, considering that in other similar studies, e.g. the Kronos Early Estrogen Prevention Study (KEEPS) conducted in women of perimenopausal age, a CAC score >50 AU was the major disqualifying cardiovascular risk factor, which was detected in 3.7% of the 1,078 women screened. As stated by the KEEP researchers, "an unexpected finding from the KEEPS screening processes was the number (nearly 4%) of women in this age group, usually considered at low risk for CVD that had to be excluded because of significant CAC, even in the absence of other clinically significant cardiovascular risk factors (i.e., elevated BMI and cholesterol, hypertension, and diabetes)"[5].

This pilot study does have some limitations. First, the small number of patients included and the absence of follow-up, do not allow us to draw any conclusion about its possible role as an independent risk factor for CVD. This limitation could easily be overcome by testing the method on a larger series and in special subgroups of patients, such as those affected by diabetes mellitus or renal impairment. Second, in our study we did not use any computed software available for the determination of calcium in a single plaque, as we did not search for a culprit lesion, but were interested in measuring the total and not the coronary calcium burden as a marker of early CA. Finally, we did not perform a systematic interobserver agreement both for the identification of intima-media tunica VC and for the determination of CALCs. However, random evaluations showed excellent reproducibility not only by expert operators, but also by advanced trainees.

Despite these limitations, our score is a promising tool to further improve the characterization of subclinical atherosclerosis due to its ability to evaluate all the principal arterial districts of the body, in a simple, repeatable and fast way. Based on these results, CALCs could be useful to complete the so-called "vascular age" evaluation, until now detectable only by IMT evaluation[26].

Further studies on larger series and with adequate follow up are necessary not only to confirm our preliminary results, but also to establish the role of this score in monitoring calcific atherosclerosis progression. Nevertheless, the validation of this score, in particular clinical settings, such as diabetes or CRF, could have a great impact on patients' prognostic stratification and follow-up.

Conclusions

CALCs may be a reliable alternative to the traditional CAC score in the evaluation of peripheral calcium load. It is highly correlated with other validated markers of subclinical atherosclerosis, such as IMT and AS and allows a better stratification of patients.

Due to the cost-effectiveness, non-invasiveness and safety, CALCs score may be useful not only for a baseline assessment of CA but also in the follow up of patients with risk factors for CVD to monitor intervention on these risk factors

For these reasons, we believe that CALCs may represent a real methodological advancement in the field of subclinical atherosclerosis compared to currently available modalities.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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